THE EFFECTS OF CULTURE MEDIA pH ON FLAGELLAR MORPHOLOGY AND MOTILITY OF *BACILLUS MEGATERIUM*

A Thesis

Presented to

The Graduate Division

Drake University

In Partial Fulfillment

of the Requirements for the Degree

Master of Arts in Biology

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by

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August 1968

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CHAPTER I

INTRODUCTION

It was almost two centuries after Leeuwenhoek's discovery of bacteria that Cohn, in 1872, first recognized the flagella of the bacterium, Spirillum volutans as true organelles. Since that time, using a variety of organisms and aided by advances in technology, extensive investigations have been carried on relative to the nature and significance of these organelles. The size, shape and distribution of the flagella on bacterial organisms were ascertained by careful staining preparations, and these characteristics have since become an important basis for bacterial identification. Since flagella were found on only free-swimming bacteria, these structures were assumed to be locomotory, however, the actual physiology of the motion is still not completely understood.

Determination of the flagellar composition has been made by growth of the organisms in nitrogen-free media, subjection to heat following antibidy adsorption, paper chromatography, X-ray diffraction studies and ultra-violet light absorption.

These studies seem to indicate the protein nature of the flagellar material, and hence, its susceptibility

to change, in varying environmental conditions such as temperature, substrate concentration and pH.

It was the purpose of the present study to determine the effects of hydrogen ion concentration on the morphology of the flagella of Bacillus megaterium and to relate induced changes to the rate of motility.

CHAPTER II

SURVEY OF THE LITERATURE

The earliest studies of flagella were, for the most part, concerned with general appearance and staining reactions of the organelles. Classification of bacteria according to the position of the flagella on the soma, their relative abundance and time of appearance from a germinating bacterial spore were typical of the work done at this time (1872-1930).[1]

Staining techniques employing the use of mordants to increase the diameter of the flagella, thus making them more visible were developed, notably by Bailey, Gray, Leifson and Casares-Gil.[2] These methods led to obvious distortions because of the precipitated mordant on the flagella, although they did not necessarily affect the wave shape characteristic of the organelle. In an attempt to estimate the width of a flagellum, Meyer assumed that the ratio between the stained flagellum and the stained

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[1]Georges Knaysi, Elements of Bacterial Cytology (Ithaca: Comstock Publishing Company, Incorporated, 1951), p. 260.

[2]H. J. Conn, Mary A. Darrow, Victor M. Emmel, Staining Procedures Used By The Biological Stain Commission (second edition; Baltimore: Williams and Wilkins Company, 1962), pp. 156-158

soma would be the same as for the untreated bacterium. Subsequent studies showed that flagellar width varied with the medium and the species and variety of the organism, but that the flagella were uniform in diameter throughout their lengths.[1,2]

The helicoidal nature of the flagella has been known for some time. Recent negative contrast electron microscopic studies of Lowy and Hanson in 1964 have determined that the organelle is a solid structure composed of tightly packed globules forming subfibrils spiralling along an inner shaft.[3] Similar structural details have been reported by Starr and Williams,[4] and by Labaw and Mosley.[5] This would seem to disprove the assertion of Pijper that

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[1]Knaysi, op. cit., p. 263.

[2]Adrianus Pijper, "The Flagella of Spirillum Volutans," Journal of Bacteriology, 57:111-117, January, 1949.

[3]J. Lowy and Jean Hanson, "Structure of Bacterial Flagella," Nature, 202:538-540, May, 1964.

[4]Mortimer P. Starr and Robley C. Williams, "Helical Fine Structure of Flagella of a Motile Diphtheroid," Journal of Bacteriology, 63: 701-706, June, 1952.

[5]Claes Weibull, "Movement" in Structure (Vol. I of The Bacteria: A Treatise on Structure and Function, ed. I.C. Gunsalus and Roger Stanier. New York: Academic Press, 1960), p. 154.

the flagella are composed of fluid slime shredding from the surface of the slime capsule, and not really entities in themselves.[1,2]

Abram, Vatter and Koffler used ghost cells and cell membrane fragments to demonstrate that the flagella are attached to the cytoplasmic membrane,[3] and that each flagellum is connected to a basal granule with a hook-like crook at its distal end.[4] These more recent electron microscopic findings settled the controversial issue of flagellar origin that had been raging since the first bacterial cytologists propounded the theory that these organelles were simply cytoplasmic protrusions through cell wall pores.[5]

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[1]Pijper, op. cit., p. 117.

[2]_____, "Evidence That Amputation of Bacterial Flagella Does Not Affect Motility," Science, 109: 379-380, April, 1949.

[3]Dinah Abram, A. E. Vatter, and Henry Koffler, "Attachment and Structural Features of Flagella of Certain Bacilli," Journal of Bacteriology, 91:2045-2068, May, 1966.

[4]_____, "Basal Structures and Attachment of Flagella in Cultures of Proteus vulgaris," Journal of Bacteriology, 90: 1337-1354, November, 1965.

[5]Knaysi, op. cit., p. 265.

One of the most important tools in bacterial cytology in recent years has been the electron microscope. Pijper's concept of the formation of flagella as a result of motion was shown to be erroneous by Hillier, Knaysi and Baker, whose colloidal film technique proved that the flagella were present on the cell as soon as the inner spore coat split.[1] The electron microscope has been used to determine the nature of some of the ultrastructures of the bacterial cell. One of the most frequently used techniques involves the lysing of the cell by use of lysozyme or some other enzyme, negative staining with potassium phosphotungstate or uranyl acetate, and shadow cast preparations of ghost cells.[2] Ordinary light microscopy is usually unsatisfactory for flagellar work with unstained organisms, but moderate success has been achieved by use of the dark field in motility studies. The phase contrast microscope helped to resolve some of the refringence difficulties encountered with the light microscope.

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[1]James Hillier, Georges Knaysi, and Richard F. Baker, "New Preparation Techniques for the Electron Microscopy of Bacteria," Journal of Bacteriology, 56: 569-576, November, 1948.

[2]Claes Weibull, "Isolation of Protoplasts From Bacillus megaterium by Controlled Treatment With Lysozyme," Journal of Bacteriology, 66:688-695, December, 1953.

Antibody absorption by flagella results in a thickening and stiffening of the structures with an agglutination effect. The flagellar antigens, specific for particular bacteria, are referred to as H-antigens. These are an important menas of classification and give a hint to the protein nature of the flagella. Pijper observed the grouping phenomenon in 1938, confirmed by Mudd and Anderson in 1941. Tomcsik has been one of the leading workers in this area. He found that flagellar antigenicity is lost in boiled cell preparations, but retained in flagells detached from the cell by mechanical agitation. Even highly dilute antisera are capable of immobilization of flagella attached to the cell.[1]

Kerridge showed that bacteria mechanically deflagellated by centrifugation were able to regenerate flagella if incubated in optimum conditions. Bacteria held at an acid pH spontaneously deflagellated, the flagella subsequently disintegrated and lost their characteristic appearance as determined by electron microscopy, and could

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[1]Weibull, "Movement" in Structure (Vol. I of The Bacteria: A Treatise on Structure and Function) op. cit., p. 156.

no longer be precipitated by centrifugation forces used for normal flagella.[1]

Bacterial flagella consist of homogeneous aggregates of protein molecules termed "flagellin."[2] X-ray diffraction studies of chemical components of flagella of some species show that the flagellin of certain strains of Bacillus subtilis and Proteus vulgaris belongs to the keratin-myosin-epidermin-fibrinogen group of fibrous proteins.[3] Qualitative and quantitative analyses of flagellar constituents showed variation from species to species with protein content estimates to 99%. Investigations of Weibull and Koffler and his associates showed a much higher percentage of nitrogen (15-16.7%) than phosphorus (0.05%).[4] Other flagellar components found were: carbohydrates (< 0.2%), lipids (0.8%), nucleic acids (<0.1%) and ash (0.0005%).

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[1]E. F. Gale, Synthesis and Organization in the Bacterial Cell, (New York: John Wiley and Sons, Incorporated, 1959), pp. 39-41.

[2]Terrence M. Joys and Ruth W. Frankel, "Genetic Control of Flagellation in Bacillus subtilis," Journal of Bacteriology, 94: 32-37, July, 1967.

[3]Weibull, "Movement" in Structure (Vol. I of The Bacteria: A Treatise on Structure and Function), op. cit., p. 156.

[4]H. Koffler, T. Kobayaski, E. Mallet, "Cysteine-Cystine Content and the Free Amino Acid Groups of Flagellin," Archives of Biochemistry and Biophysics, 64:509, October, 1956.

Most of the amino acids except the heterocyclic compounds, tryptophan, histidine, proline and hydroxyproline were found. The molecular weight of the flagellar subunits was estimated at about 20,000.[1]

The work of Hoeniger in 1965 demonstrated that there was a direct relation between the general morphology of the flagella of Proteus mirabilis and the pH at which the organisms were cultured. Her research also indicated some differences with respect to motility as a result of this morphogenesis.[2]

In view of their proteinaceous composition, it is reasonable to hypothesize that the flagellar material would react to environmental alterations much as a pure protein would, and so be directly affected by the pH of the culture medium, either by denaturation of the constituent proteins, or in some other more subtle way.

Although much has already been done with the flagella, much still remains. The physics of locomotion is still unknown, and the influence of nuclear elements on flagellar production remains obscure.

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[1]H. Koffler, T. Kobayaski, G. E. Mallet, op. cit., p. 509.

[2]Judith Hoeniger, "Influence of pH on Proteus Flagella," Journal of Bacteriology, 90: 275-277, July, 1965.

CHAPTER III

MATERIALS AND METHODS

The organism used in this investigation was a strain of Bacillus megaterium maintained in the microbiology laboratory at Drake University. This bacteria culture did not differ appreciably from the standard description as given in Bergey's Manual of Determinative Bacteriology, 7th Edition. For staining and hanging drop preparations used in this study, the bacteria were cultured in 16 mm. tubes of tryptic soy broth (Difco) at 35° C for 24-48 hours. The pH of the culture medium was adjusted by dropwise addition of 0.1 N NaOH or dilute HCl within a range of 6.0 to 9.5.

Slides to be used for staining bacterial smears were allowed to soak in a 5:1 mixture of concentrated sulfuric acid and distilled water for a period exceeding twenty-four hours. They were then drained, rinsed for approximately one minute in distilled water, 95% ethanol and flamed. When the slides were cool, they were streaked by applying a loopful of the bacterial inoculum to their tilted surfaces, allowing the media to run the length of the slides. The slides were then air dried for a minimum of two hours and stained, using a modified Swatek adaptation of the Leifson flagella stain.

Leifson Flagella Stain[1]
Swatek Adaptation

Solution I

$KAl(SO_4)_2 \cdot 12\ H_2O$ (sat. aqueous sol'n.)	20 ml.
Tannic Acid (20% aqueous)	10 ml.
Distilled Water	10 ml.

Solution II

Ethyl Alcohol (95%)	15 ml.
Basic Fuchsin (sat. in 95% ethanol)	3 ml.

Solutions I and II were stored separately in a refrigerator at approximately 5° C, mixed and triple-filtered just prior to use. Approximately 0.5 ml. of stain was flooded over the surface of the dried smear and allowed to remain undisturbed for approximately 90 seconds, rinsed in a gentle stream of tap water, air dried, and observed using the 97X oil immersion objective and a 10X ocular (Bausch and Lomb Model STA.).

Semi-solid agar (0.5% Difco Bacto-Agar in tryptic soy) was used for cultivation of the organism to determine its relative motility under differing pH conditions. The Eklund and Lankford adaptation of the motility medium was adjusted for pH by the addition of NaOH or dilute HCl as

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[1]Frank E. Swatek, Textbook of Microbiology, (Saint Louis, Missouri: C. V. Mosby Company, 1967), p.71.

above, and incubated for forty-eight hours at 30° C. Pyrex brand 10 mm. petri dishes were used for this culture. The path of the bacilli through the medium was marked by a pink halo due to the reduction of 2,3,5-triphenyltetrazolium by the growing bacteria.

Semi-solid Motility Medium[1]
Eklund-Lankford Adaptation

Nutrient broth (Difco tryptic soy)	100 ml.
Agar (Difco Bacto-Agar)	0.5 %
2,3,5-triphenyltetrazolium chloride	0.001 %

Photographs of both the stained bacilli and the petri plate cultures were made by Mr. Anthony M. Kuzma, professional medical photographer at Marquette University School of Medicine in Milwaukee, Wisconsin, using a Leitz Panphot with carbon arc light source. A 90X oil immersion objective with Apochromat lens for color correction and a 10X ocular were used, giving a total magnification of 1500X for the organisms, while the macro-setup for the petri plates required a Microtessar lens.

The relative motility of the living bacteria was studied by preparing standard hanging drop preparations and observed using the 43X high power objective and a 10X ocular. The approximate time observed for a single bacterium

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Eklund and Lankford, Laboratory Manual for General Microbiology (Englewood Cliffs, New Jersey: Prentice Hall, Incorporated, 1967), p. 278.

to traverse the radius of the high power field (0.25 mm.) was determined by actual stopwatch-clocking. Results were tabulated and plotted on a graph. In all cases except one, fifty such clockings were made. Due to the paucity of organisms at pH 9.5, and the fact that they were barely motile, it was possible to make only a limited number of readings. All cultures studied were twenty-four populations.

CHAPTER IV

RESULTS AND INTERPRETATION OF DATA

This study was designed to show that the pH of the culture medium influences the motility rate of Bacillus megaterium and also to show possible morphological alterations in the flagellar pattern typical of this organism when cultured under ideal conditions.

In order to study the change in flagellar structure, Swatek's adaptation of the Leifson flagella stain was used on bacterial smears made from cultures grown at differing pH levels. Photomicrographs of these organisms comprise the data presented for comparison.

Motility studies were conducted in two ways. One method involved growth of B. megaterium through semi-solid motility medium adjusted to the desired pH by the addition of NaOH or HCl. Photographs showing the relative growth distributions on these plated media were then taken.

A second method utilized direct observations of individual bacteria traversing the 0.25 mm. radius of the high power field of a light microscope (43X) with a 10X ocular, and the tabulation of approximate excursion times. The relative rates of the organisms cultured at different pH levels were compared.

In each case, B. megaterium grown at pH 7.3 were considered the norm against which similar organisms raised at differing pH's were compared.

Figures 1 and 2 show B. megaterium cultured in tryptic soy broth (Difco), at pH 7.3. The flagella of these organisms were long and kinky, averaging five to six waves each.

Bacteria cultured at pH 6.0 bore flagella that in most cases were shorter than those of the organisms raised in the reference medium (pH 7.3), and irregularities in the amplitude and length of the waves were shown. Instead of the regular flection amplitude and wave length patterns characteristic of the flagella of the bacilli cultured at pH 7.3, the flagella of the organisms grown in the more acid medium deviated considerably from them, having both deeper and more shallow waves, as well as a greater variation in distance from one wave crest to the next.

At pH 8.5, the flagella of the organisms were slightly reduced in length with the kinks less abberent than those of the bacilli grown at pH 6.0. Figure 5 is a photomicrograph of a stained smear of B. megaterium on which a slight tendency toward straightening of the flagella can be noted when compared with these organelles on bacteria cultured at pH 7.3 (Figures 1 and 2).

The flagella of the bacteria cultured in the medium of pH 9.0 showed an increased tendency toward straightening over that noted in the medium of pH 8.5. At pH 9.0 there is a conspicuous lack of the otherwise characteristically kinky normal flagella, and they were, in fact, almost straight. Figure 6 is a microphotograph of B. megaterium cultured at pH 9.0 and prepared with Leifson flagella stain.

Figure 7 represents bacilli grown at pH 9.5 and shows several well-defined bacteria, but there is no evidence of flagella.

Assuming that bacterial flagella are locomotory organelles, and that the morphology of these structures is altered by a change in the pH of the culture medium, it was deemed reasonable to relate the pH of the medium with the relative rate of motility of the organism. One seim-quantitative estimate of the relative motility of B. megaterium was the use of semi-solid motility medium (o.5% Difco Bacto-Agar in tryptic soy broth) through which the bacilli are able to move. Figures 8 through 11 show the relative growth of the organism in the 0.5% agar medium adjusted to varying pH levels and incubated for forty-eight hours. The bacteria grew well at all levels of pH within the range tested.

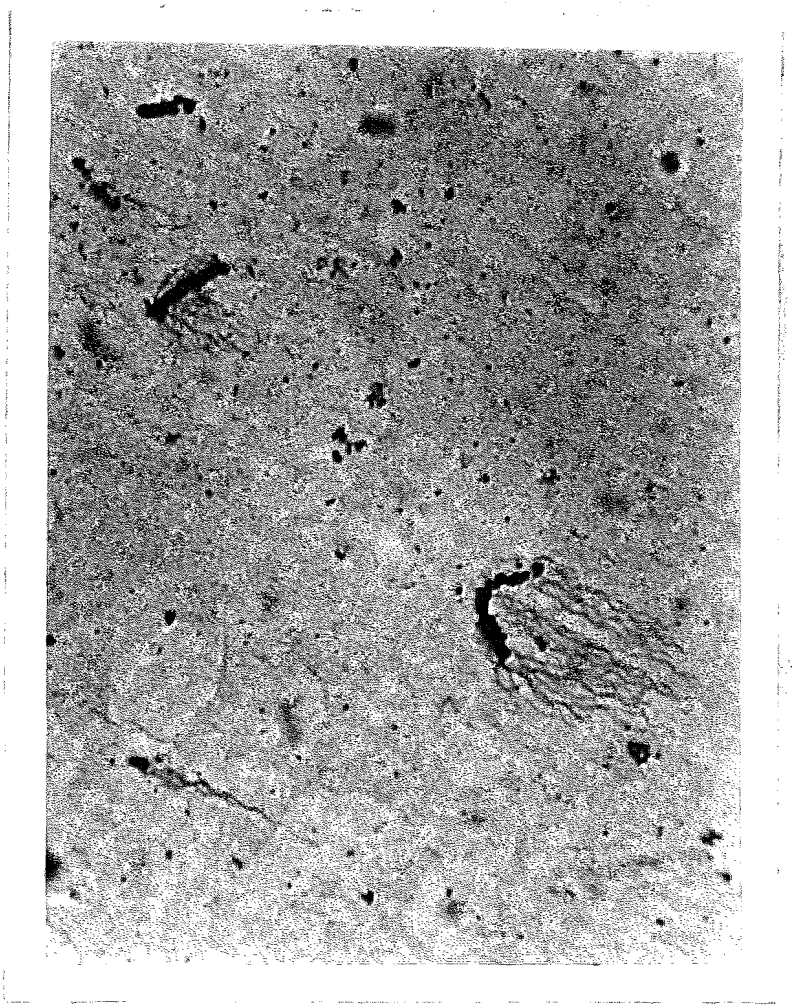

Figure 1. B. megaterium cultured in tryptic soy broth, pH 7.3, stained with Leifson flagella stain (1500X).

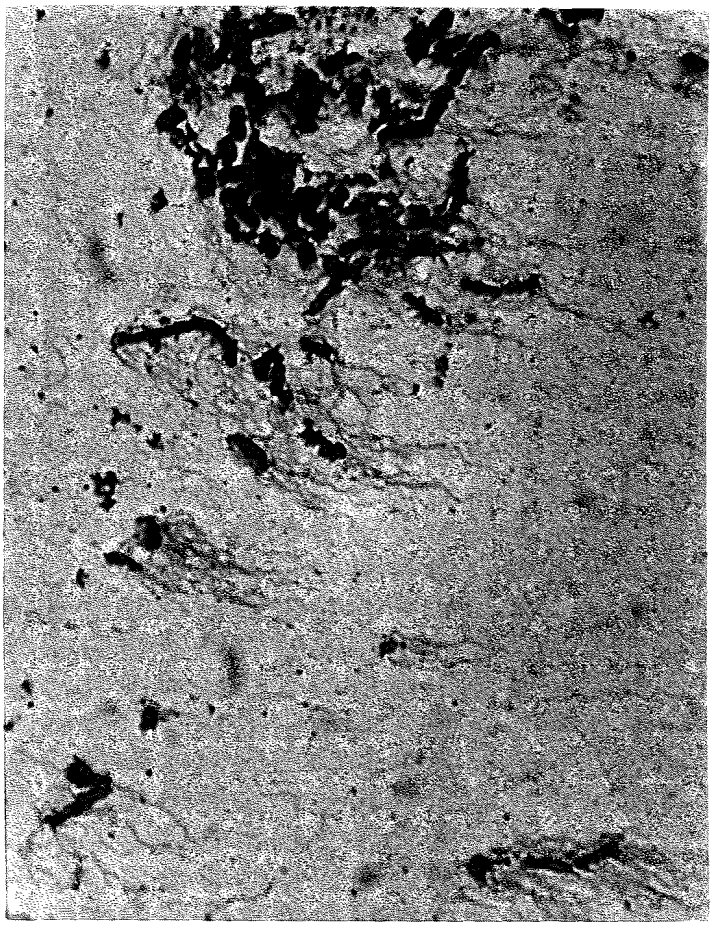

Figure 2. B. megaterium cultured in tryptic soy broth, pH 7.3, stained with Leifson flagella stain (1500X).

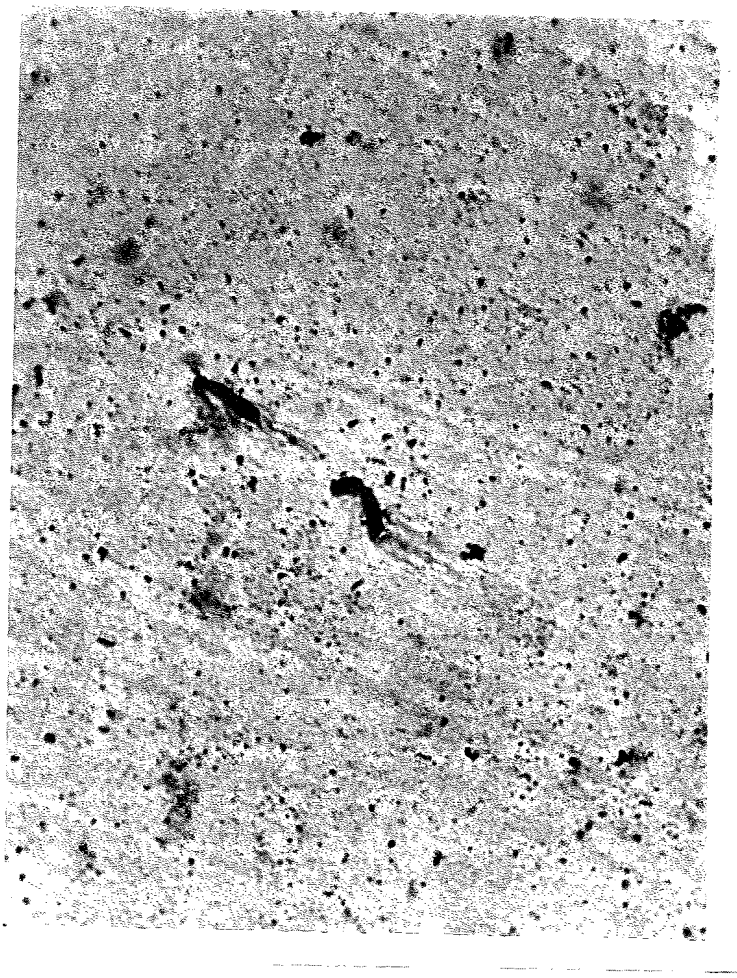

Figure 3. B. megaterium cultured in tryptic soy broth, pH 6.0, stained with Leifson Flagella stain (1500X).

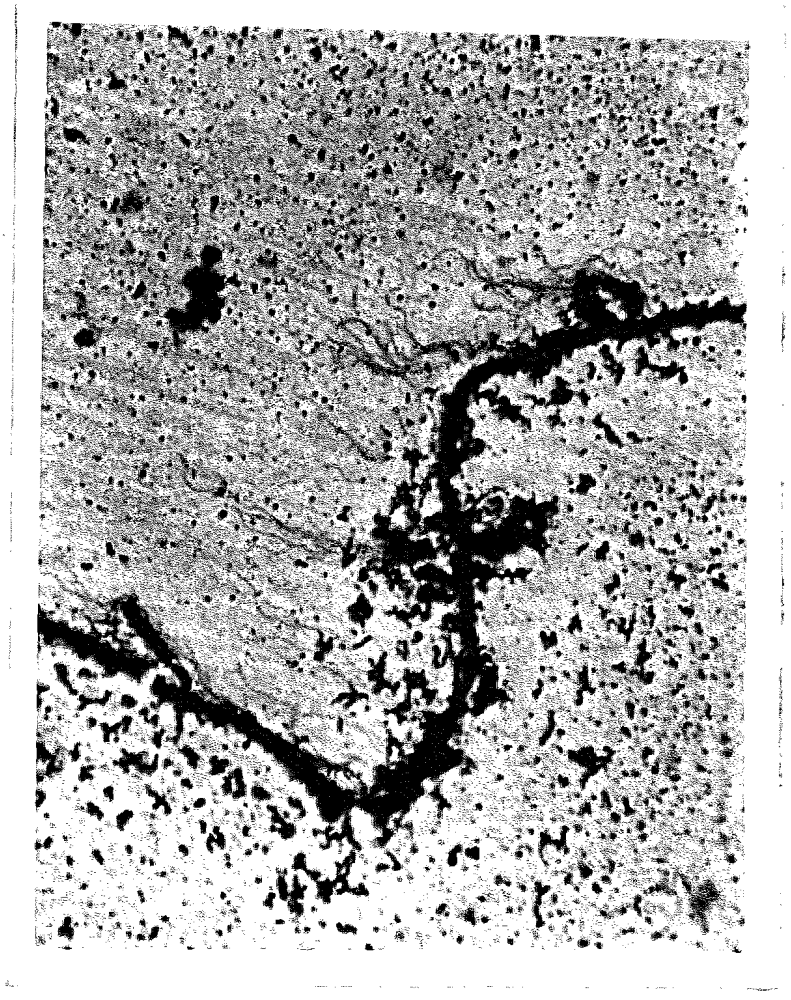

Figure 4. B. megaterium cultured in tryptic soy broth, pH 6.0, stained with Leifson flagella stain (1500X).

Figure 5. B. megaterium cultured in tryptic soy broth, pH 8.5, stained with Leifson flagella stain (1500X).

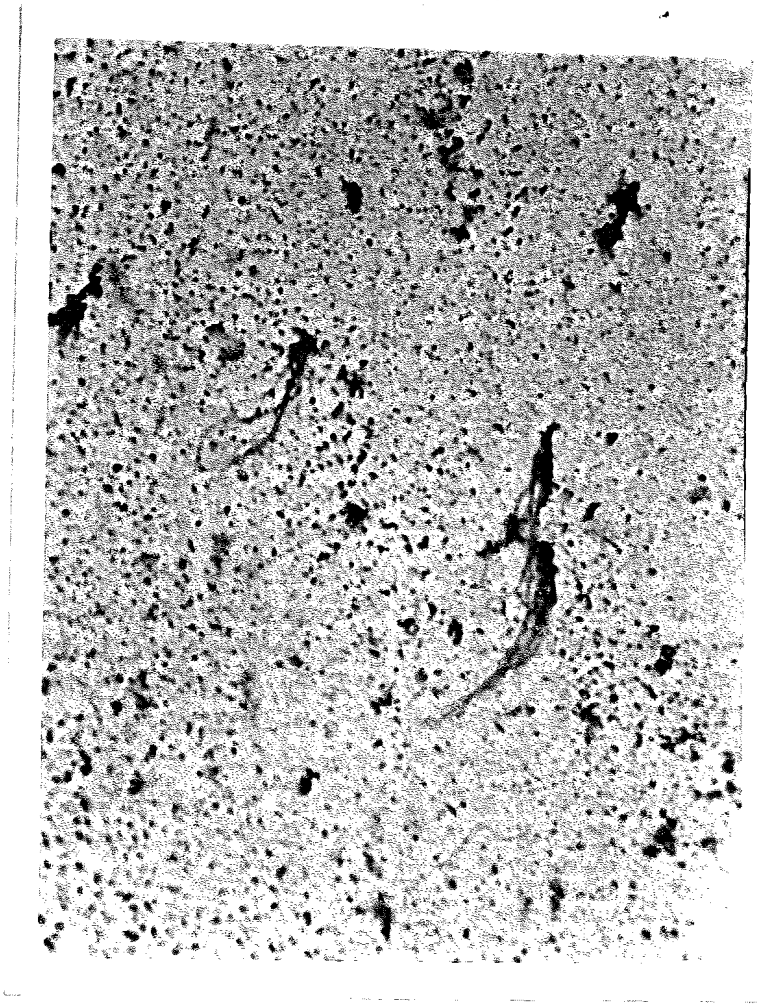

Figure 6. B. megaterium cultured in tryptic soy broth, pH 9.0, stained with Leifson flagella stain (1500X).

Figure 7. B. megaterium cultured in tryptic soy broth, pH 9.5, stained with Leifson flagella stain (1500X).

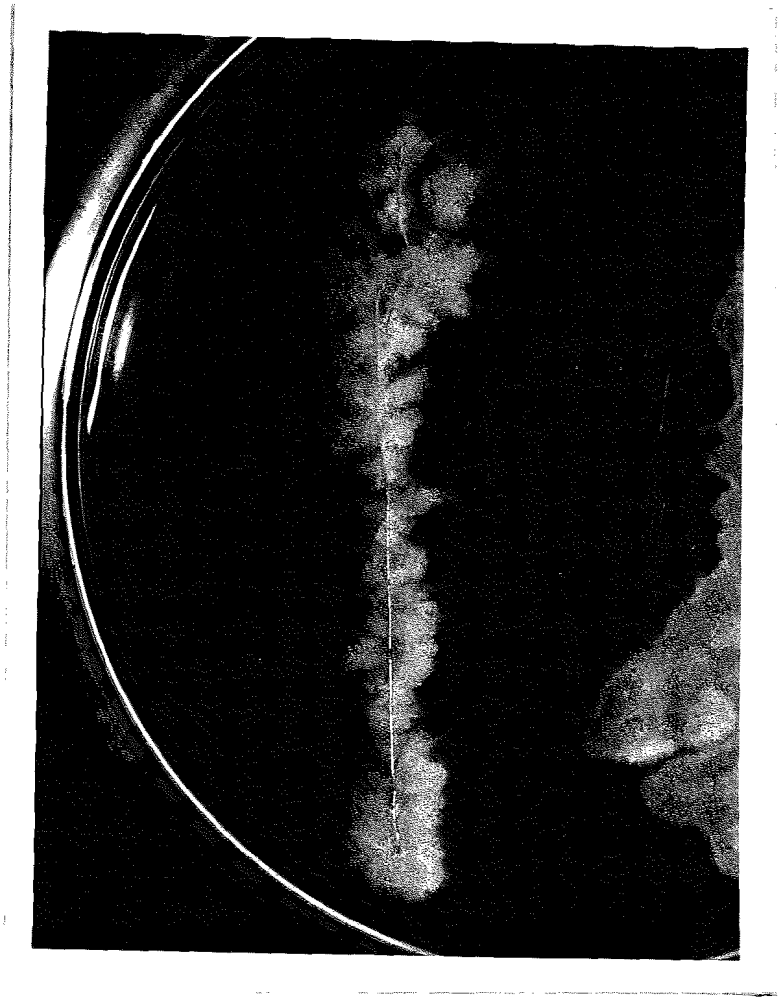

Figure 8. B. megaterium cultured on semi-solid motility agar, pH 6.5, for forty-eight hours.

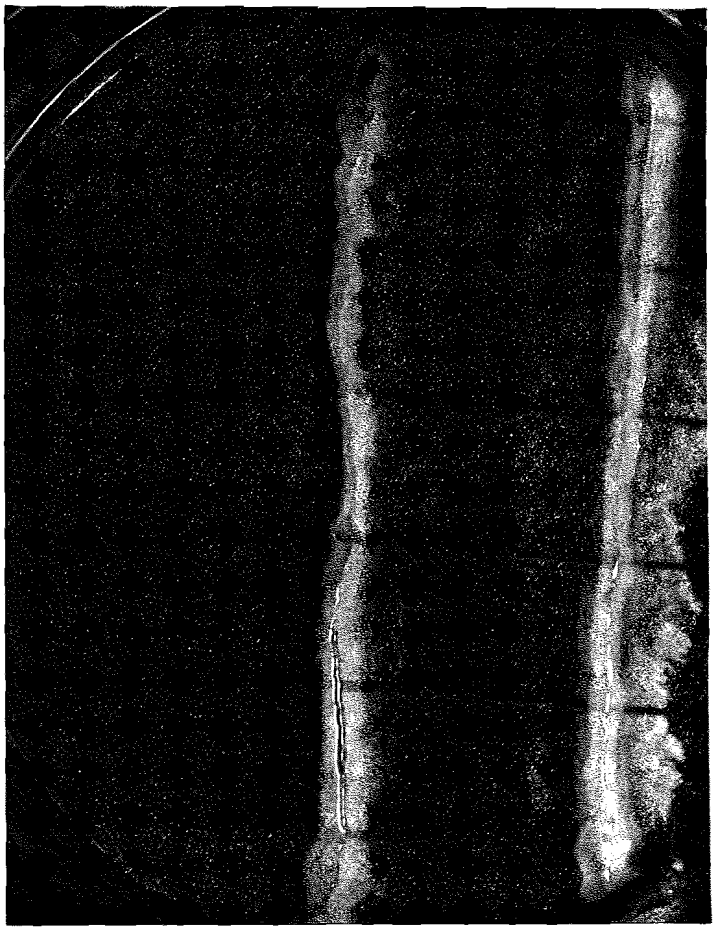

Figure 9. B. megaterium cultured on semi-solid motility agar, pH 7.3, for forty-eight hours.

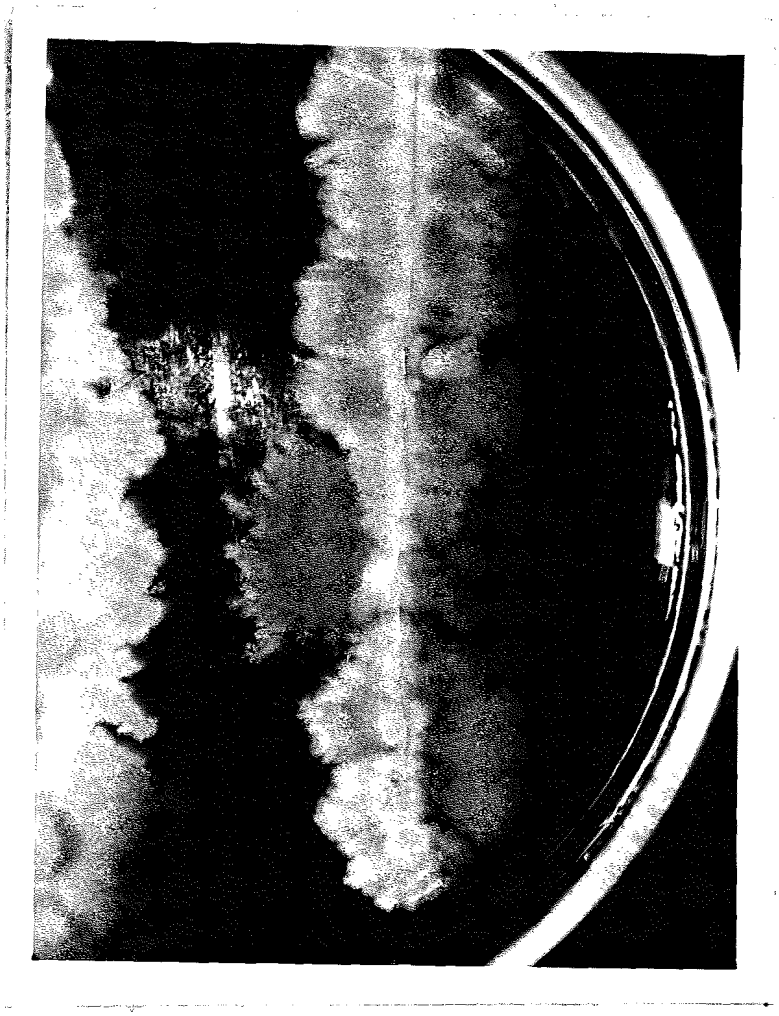

Figure 10. B. megaterium cultured on semi-solid motility agar, pH 8.5, for forty-eight hours.

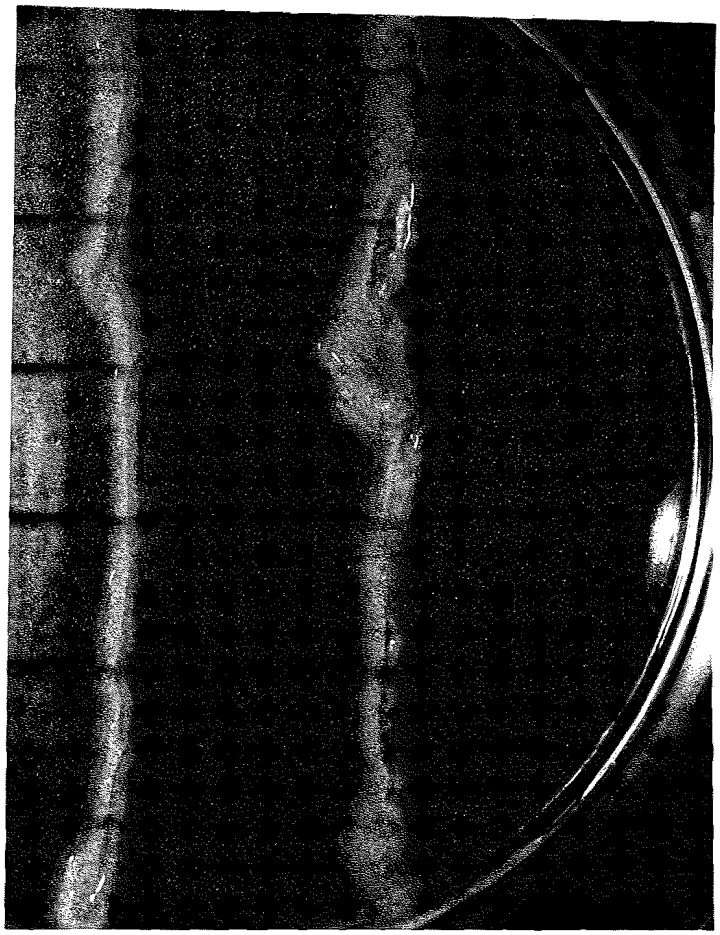

Figure 11. B. megaterium cultured on semi-solid motility agar, pH 9.0, for forty-eight hours.

Figure 10 shows the growth of B. megaterium cultured in semi-solid agar adjusted to pH 8.5. Since the growth of the organism at this level radiates the farthest from the line of inoculation, it can be assumed that these bacilli are the most actively motile. The growth of the less motile organisms was denser along the line of inoculation, and restricted to a relatively narrow radius. The growth patterns of these less motile bacteria are illustrated in Figures 8, 9, and 11.

Another semi-quantitative estimate of relative motility was made by direct observation of individual living bacilli examined in standard hanging drop preparations. Stopwatch readings for the length of time required for each of 50 bacteria cultured at a given pH, to traverse the 0.25 mm. radius of the 43X high power field were recorded in Table I. Because of the paucity of organisms at pH 9.5, and their apparent immobility, only a few readings could be made.

Figure 12 shows a comparison of pH with the mean time in seconds which was observed as needed for a bacterium in a hanging drop to traverse the radius of the 0.25 mm. high power field of the light microscope. 95% confidence limits are also indicated for each mean. Figure 13 depicts the mean rate in mm. x 10^{-2}/sec., for the readings corresponding to the data presented in Figure 12.

TABLE I

NUMBER OF SECONDS REQUIRED FOR ONE BACILLUS MEGATERIUM ORGANISM TO TRAVERSE THE 0.25 mm. RADIUS OF A 43X FIELD OF A LIGHT MICROSCOPE AS OBSERVED IN HANGING DROP PREPARATIONS

pH 6.5		pH 7.3		pH 8.5		pH 9.0		pH9.5
6.0	11.0	4.0	8.0	4.2	6.4	6.0	9.0	122.2
6.8	11.2	4.0	8.0	4.8	6.4	6.2	9.0	124.6
	11.4	4.8	8.0	4.8	6.4	6.2	9.0	
7.4	11.4		8.0	4.8	6.4	6.4	9.0	134.0
	11.6	5.0	8.2	4.8	6.4	6.6	9.0	136.6
8.0	11.8	5.0	8.2		6.6	6.8	9.0	138.2
8.4		5.0	8.4	5.0	6.6	6.8	9.0	
8.4	12.8	5.2	8.6	5.0	6.8		9.2	146.8
8.6	12.8	5.4		5.0		7.0	9.2	
8.6		5.4	9.0	5.0	7.0	7.0	9.2	150.4
	13.0		9.0	5.2	7.0	7.2	9.4	154.8
9.0	13.6	6.0	9.0	5.2	7.0	7.4	9.6	
9.0	13.8	6.0	9.2	5.2	7.0	7.8	9.8	168.8
9.2		6.0	9.2	5.4	7.0	7.8	9.8	
9.2	14.0	6.0	9.2	5.8	7.2			186.4
9.2	14.0	6.0		5.8	7.2	8.0	10.0	
9.8	14.0	6.0	10.0	5.8	7.2	8.0	10.2	
	14.0	6.2			7.4	8.0	10.4	
10.0		6.4	11.0	6.0	7.6	8.4	10.6	
10.0	15.0	6.4	11.8	6.0	7.8	8.4		
10.0	15.2	6.8		6.0	7.8	8.4	11.2	
10.2			12.8	6.2	7.8	8.4	11.2	
10.2	16.0	7.0		6.2		8.6	11.2	
10.2	16.2	7.0	13.0	6.2	8.4	8.6		
10.2		7.4	13.0	6.2		8.6	12.0	
10.6	17.0	7.4	13.0	6.2	9.2	8.8	12.2	
10.8	17.4	7.6	13.0				12.2	
	17.8	7.8	13.8		11.0			
		7.8					14.0	
	18.2		16.2		12.4			
	18.8						21.0	
	22.4							
	25.6							

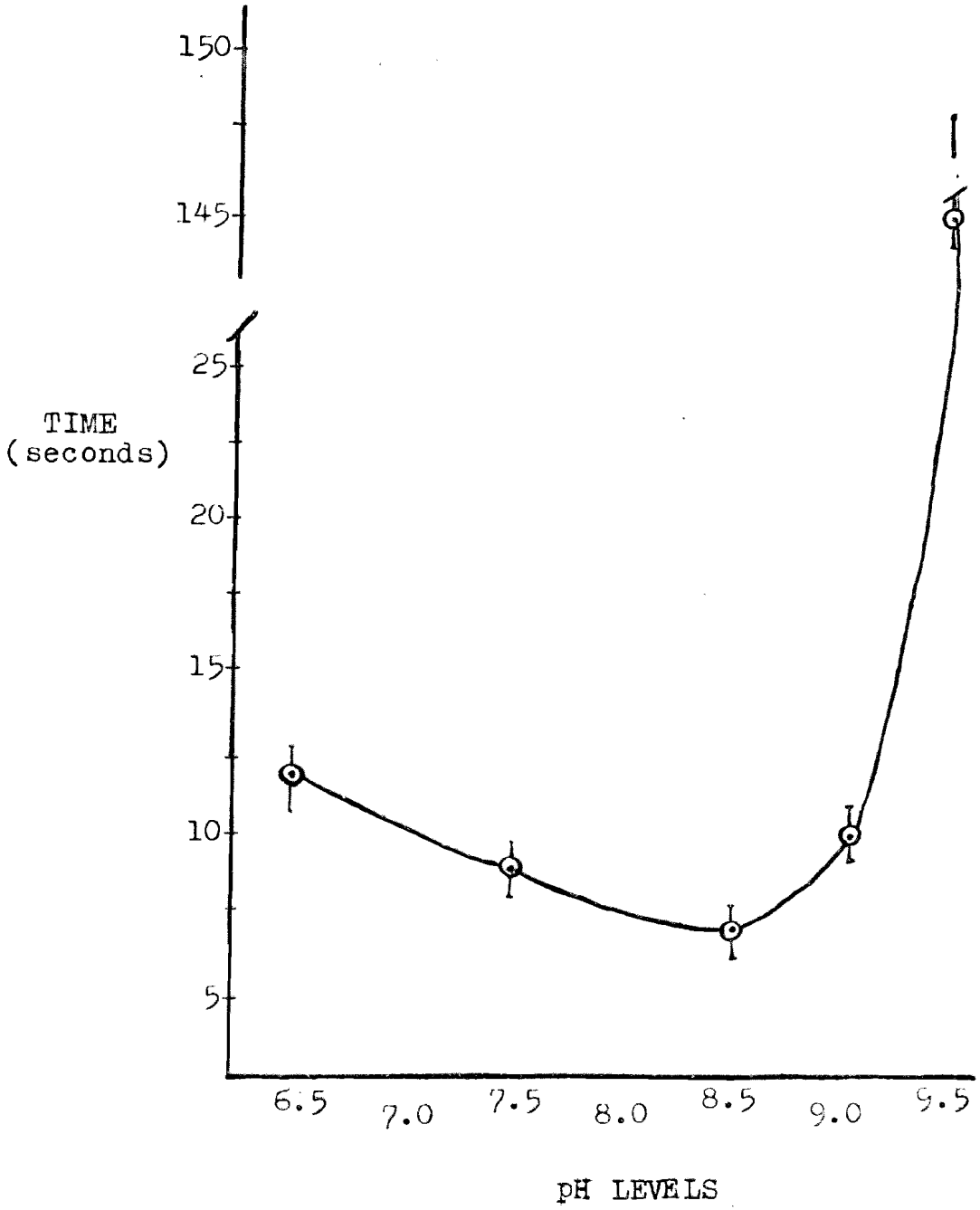

Figure 12. Mean time for one B. megaterium cell to traverse the radius of a high power objective as observed in hanging drop preparations at varying pH levels.

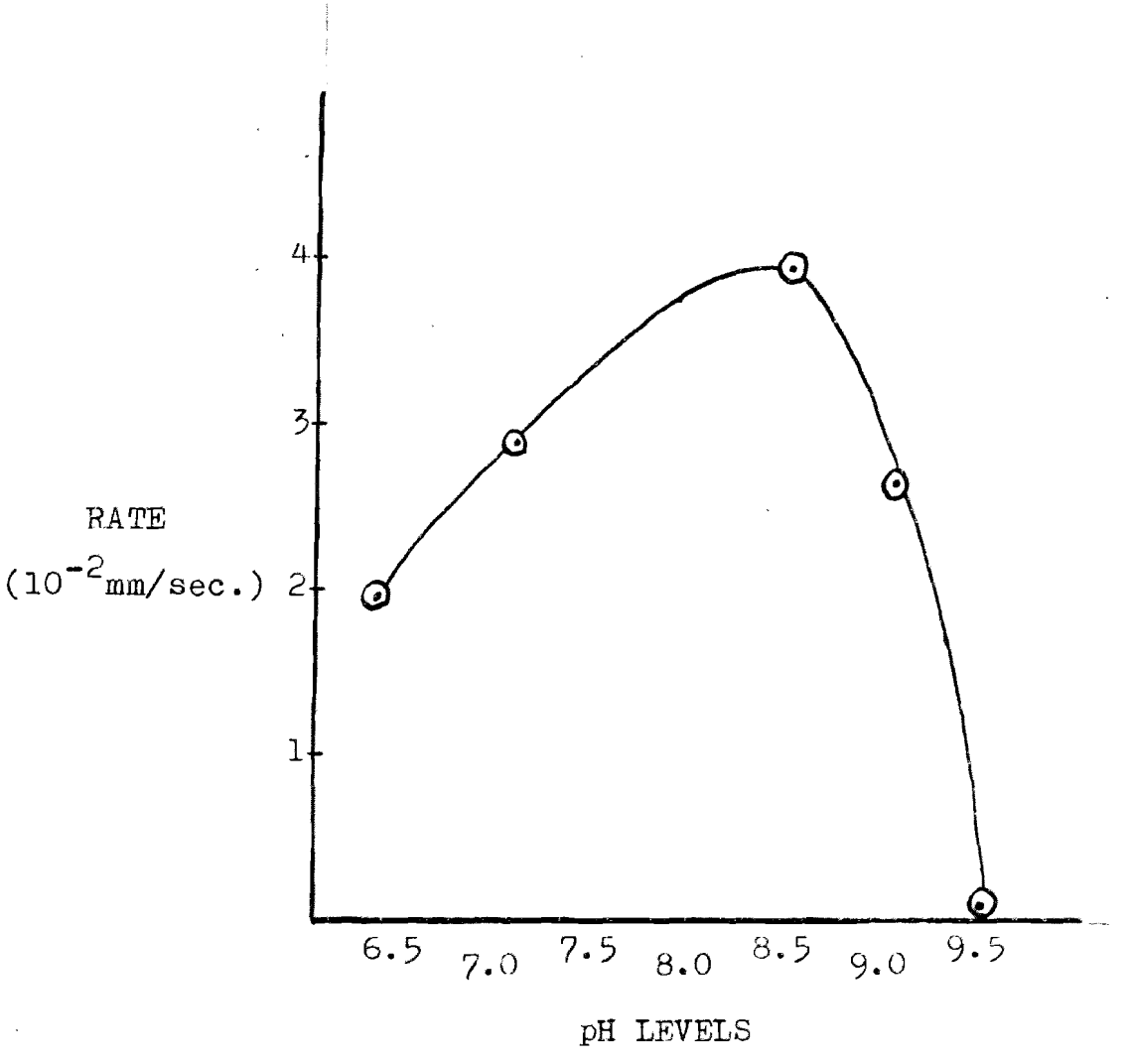

Figure 13. Mean rate of one B. megaterium cell in traversing the radius of a high power field as observed in hanging drop preparations at varying pH levels.

Table II presents a summary of the data, including a t-score to test the significance of the differences in rates.

TABLE II

Mean Time and Rate of One B. megaterium Organism in Traversing the 0.25 mm. Radius of a 43X Field of a Light Microscope as Observed in a Hanging Drop Preparation

pH	Mean time (sec.)	σ	Mean rate ($\times 10^{-2}$ mm/sec)	t-score
6.5	12.29	3.93	2.03	
				15.17
7.3	8.33	2.74	3.00	
				8.68
8.5	6.57	1.55	3.78	
				12.25
9.0	9.17	2.52	2.71	
				2736.60
9.5	146.0	18.14	0.17	

Graphic representation of the datta summary is shown on Figures 12 and 13. As the pH of the medium increases from pH 6.5 to pH 8.5, the point corresponding to the lowest mean time, there is almost a two-fold decrease in the mean time observed for the radial excursion of a bacterium. Fom pH 8.5 to pH 9.0 there is a moderate increase in the observed time, and by pH 9.5, the lowest mean time is increased by a factor of 20. Graphically represented, the least mean time observed at pH 8.5, is the lowest point on Figure 12, and the greatest corresponding mean rate as shown on Figure 13.

Because of their protein nature, it is reasonable to assume that the flagella of Bacillus megaterium would, in some way, be affected by a change in the hydrogen ion concentration of the culture medium. This study has shown that although the organism itself can tolerate a wide pH apectrum, the locomotory organelles exhibit morphological variation through the pH range of this study. It will be noted from the photographs (Figures 5 and 6), that Bacillus megaterium cultured at pH 8.5 possess flagella shorter than those of bacteria raised at pH 9.0. The organisms cultured at pH 8.5, however, are more actively motile. This would indicate that there is an optimum flagellar length-speed ratio. At the alkaline levels where the flagella are long and tend to mat, as indicated by the formation of a thick pellicle (pH 9.0) in broth, it is possible that this decrease in motility is due to a simple mechanical entanglement of the elongate flagella. Bacteria cultured at pH 6.0 possessed flagella comparable in length to those raised at pH 8.5, but the organisms raised in the acid medium were markedly less motile. It could be hypothesized that some chemical interference is involved.

More significant than length, is the variability of degree of flection exhibited by the flagella. There is a trend toward decreased kinkiness as the medium becomes less acid. At the point where this straightening is most noticeable, pH 9.0, motility is appreciably diminished, indicating that there is an optimum flagellar wave amplitude-speed ratio as well. Increasing the pH to 9.5 results in a near total immobilization of the bacteria and an apparent loss of flagella. Electron microscope studies by Hoeniger with Proteus mirabilis showed similar results.[1]

Those bacilli cultured at pH 9.5 appear to have lost their flagella completely (Figure 7). Two factors could have been operative to explain this phenomenon: (1) the alkaline medium may have so weakened the flagella that they fractured upon contact with the slide, however there are no free flagella apparent to give visual evidence of this possibility; (2) the basic dye (basic fuchsin) used in the Leifson staining method would have less affinity for the bacteria at an alkaline level, although the bacteria that did take up the stain were sufficiently dark.

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[1]Judith Hoeniger, Influence of pH on Proteus Flagella," Journal of Bacteriology, 90: 275-277, July, 1965.

Growth patterns in broth varied with the change in hydrogen ion concentration of the media. Both extremes of the pH spectrum used in this study supported only sparse populations which, in both cases, were restricted to a slight turbidity at the bottom of the tubes. Organisms grown in the range between pH 6.5 and 7.5 were uniformly dispersed throughout the media, and produced a moderate turbidity in each case. At pH 9.0, however, there was a sharp decline in the optical density of the culture, and a heavy pellicle formed within twenty-four hours. In all cases, the turbidimetric estimates were done without instrumentation, but were considered definitive enough that more refined measures were deemed unnecessary.

Motility studies that were conducted in two ways, growth of B. megaterium through semi-solid motility agar and direct microscopic observation of living bacilli in hanging drop preparations, showed that motility was greatest at pH 8.5 and least at both extremes of the pH range considered.

A statistical comparison of the rates of motility summarized in Table II and depicted graphically on Figures 12 and 13, indicate that these rates vary significantly when comparing hydrogen ion concentrations studied and are within the one per cent level of significance as determined by the t-score.

From the foregoing studies, it is quite apparent that there is a direct relation between the motility of Bacillus megaterium and the hydrogen ion concentration of the medium in which the organisms are cultured. It is also quite clear that there is some morphological change effected by such pH variation. This study is, by no means conclusive, but only suggestive of a number of avenues of investigation that could be followed employing different techniques and methods with other organisms.

CHAPTER V

SUMMARY

1. This study was designed to show that the pH of the culture medium influences the motility rate of Bacillus megaterium and also to show possible morphological alteration in the flagellar pattern typical of the organism cultured under ideal conditions.

2. The organism was cultured in tryptic soy broth and in semi-solid motility agar medium adjusted to varying pH levels by the addition of NaOH or HCl.

3. Leifson flagella stain preparations were made from smears of the broth cultures and examined for any change in flagellar morphology.

4. Observation showed that there is an increased straightening of the flagella at pH 9.0.

5. Motility rates can be compared by cultivation of B. megaterium in semi-solid agar at varying pH's and by hanging drop observations.

6. The bacteria having greatest motility will travel the farthest from the line of inoculation in a forty-eight hour period when raised in semi-solid motility agar. Observation showed that, by this criterion, bacteria cultured at pH 8.5 were the most motile.

7. Stopwatch timing of bacteria in hanging drop preparations in culture media of various pH values as they traversed the radius of a high power field were made. Organisms grown at pH 8.5 showed the greatest rate of movement.

8. The pH of the growth medium affects both the morphology and the motility of Bacillus megaterium by altering the wave amplitude of their locomotory organelles, the flagella.

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